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TITLE: Proteolytic Regulation of the Intestinal Epithelial Barrier: Mechanisms and Interventions

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14. ABSTRACT Inflammatory bowel diseases are characterized by chronic, progressive and destructive inflammation of the gastrointestinal tract. The two main forms of inflammatory bowel diseases (IBD), Crohn's disease and Ulcerative Colitis, currently affect over 1 million Americans including military personnel, and the incidence among aging veterans is rising. Compromised intestinal barrier function underlies much of the pathology associated with many inflammatory bowel diseases. Matriptase is a membrane-anchored serine protease encoded by the <i>Suppression of Tumorigenicity-14 (ST14)</i> gene that is required for epithelial barrier homeostasis. The project uses the <i>St14</i> hypomorphic mouse model of matriptase deficiency to 1) determine molecular mechanisms that mediate matriptase protection during DSS-induced experimental inflammatory colitis, 2) define molecular mechanisms by which matriptase becomes decreased during inflammatory colitis, and 3) investigate the importance of matriptase to cytokine induced barrier loss using an <i>in vitro</i> model of intestinal barrier repair. Our data demonstrate that the matriptase barrier forming pathway is down-regulated at the transcriptional and protein levels by cytokines produced during inflammatory colitis. Further, matriptase acts downstream of prostasin to mediate barrier formation and both of these proteases are coordinately regulated. The loss of this pathway is likely to facilitate intestinal barrier disruption in human IBD.						
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INTRODUCTION:

Inflammatory bowel diseases (IBD) are characterized by chronic, progressive and destructive inflammation of the gastrointestinal tract. The two main forms of IBD, Crohn's disease and Ulcerative Colitis, currently affect over 1 million Americans including military personnel, and the incidence among aging veterans is rising. Compromised intestinal barrier function is believed to underlie much of the pathology of IBD. Matriptase is a membrane-anchored serine protease encoded by *Suppression of Tumorigenicity-14* (*ST14*) that is required for epithelial barrier homeostasis. Here, we are investigating matriptase dysregulation and its contribution to the pathogenesis of acute colitis using the *St14* hypomorphic mouse model of matriptase deficiency. Matriptase expression and regulation are being studied using the *St14* hypomorphic mouse and matriptase dysregulation is being investigated by subjecting *St14* hypomorphic and control mice to an experimental model of inflammatory colitis. The goal of the proposed studies is to determine the mechanisms by which matriptase protects gut barrier function in inflamed mucosa. The specific aims are (1) to determine the molecular mechanisms that mediate matriptase protection during experimental colitis by defining the effects on immune responses and epithelial cell regeneration and repair, (2) to define mechanisms responsible for down-regulation of matriptase in inflamed mucosa, and (3) to determine the contribution of matriptase loss to barrier dysfunction induced by cytokines associated with IBD pathogenesis.

KEYWORDS:

Intestinal barrier

Intestinal permeability

Crohn's disease

Inflammation

Inflammatory bowel disease

Interleukin 4

Interleukin 13

Intestinal epithelial cells

Matriptase

Prostasin

Serine Protease

Type II transmembrane serine protease

Ulcerative colitis

OVERALL PROJECT SUMMARY:

Research progress is aligned with respect to each task outlined in the SOW:

TASK 1: *Seek regulatory approval for use of animals which includes the IACUC, University of Maryland, and the USAMRMC ACURO. Prior approval will also be obtained prior to any modifications of the protocol (months 1-36).*

An updated IACUC protocol was approved by the IACUC of the University of Maryland, Protocol #0913001 (expiration date 10/20/2016) and the most recent annual report approved on 09/19/2014. The updated protocol was submitted to USAMRMC ACURO for approval by Sheron Westbrook of the USAMRMC Animal Care and Use Review Office prior to 01/27/2014.

TASK 2: *To determine the molecular mechanisms that mediate matriptase protection during mucosal inflammation in experimental colitis. (months 1-36).*

OBJECTIVE: To define mechanisms by which the intestinal epithelial barrier is protected by matriptase during inflammatory colitis.

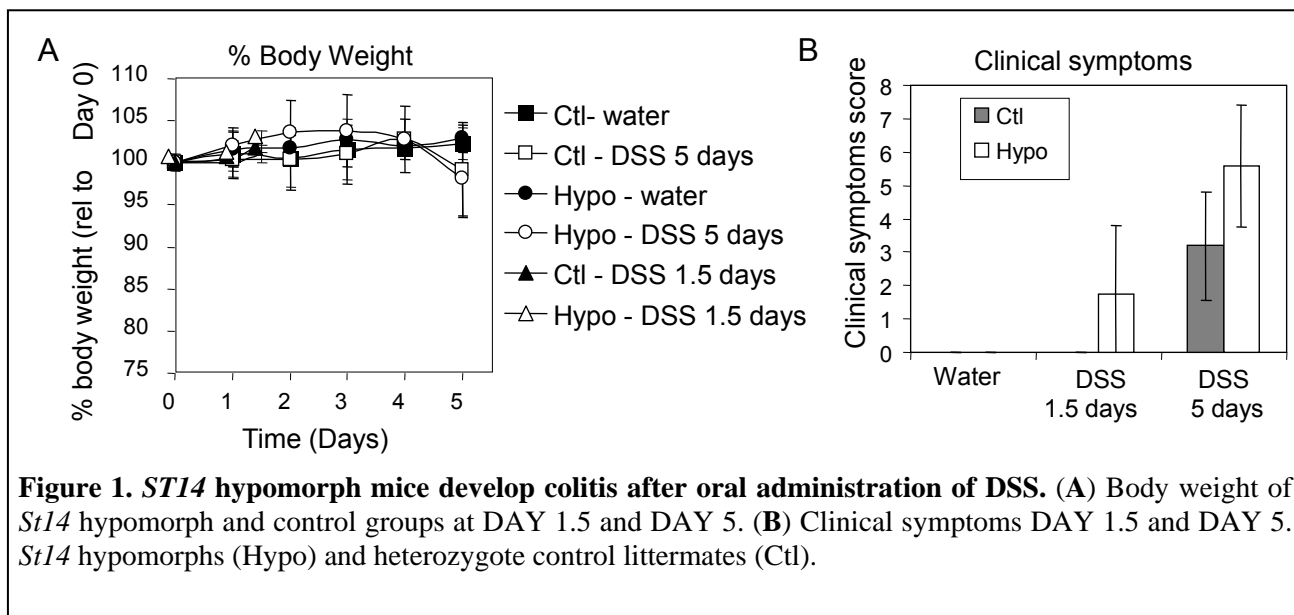
We hypothesize that the intrinsic defect in the intestinal barrier associated with the failure to form functional tight junctions in *ST14* hypomorphic mice (1; 2) prevents gut barrier repair and therefore perpetuates destructive intestinal inflammation associated with colitis and IBD, whereas normal matriptase levels in control mice during recovery enables essential barrier repair that promotes colitis resolution. Our experiments test this hypothesis utilizing a standard experimental mouse model of DSS-induced colitis. In this colitis model, the initiating trigger, DSS, causes injury to the epithelial layer provoking activation of an innate immune response to luminal contents and the induction of barrier repair processes. The experimental tasks are designed to investigate whether the increased permeability of *ST14* hypomorphic intestinal epithelium causes recurring priming of the immune system with increased immune cell infiltration and activation, and/or whether diminished matriptase increases epithelial cell destruction and/or interferes with mechanisms of epithelial cell regeneration and repair.

The specific tasks are:

(a) To induce colitis in groups of ST14 hypomorph and control littermate mice by administration of DSS in drinking water, and then replace with water only. Mice will be monitored for body weights, survival and clinical symptoms (months 1-36).

Year 1 summary - This task requires experimental *St14* hypomorphic mice and control littermate mice obtained from our IACUC approved breeding protocol 0113002. During year 1, we encountered problems due to unusually low breeding likely caused by construction of a building near the animal facility. This problem was resolved towards the end of year 1 and initial studies were completed. Preliminary analysis of available mice treated with 2% DSS in drinking water for 7 days to induce colitis showed a) *St14* hypomorphic mice lost weight much more rapidly than their control littermates after DSS treatment, and b) *St14* hypomorphic mice showed enhanced symptoms of colitis evidenced by a modest reduction in colon length, and more severe symptoms of clinical disease. These data suggest that matriptase deficiency in mice enhances disease severity.

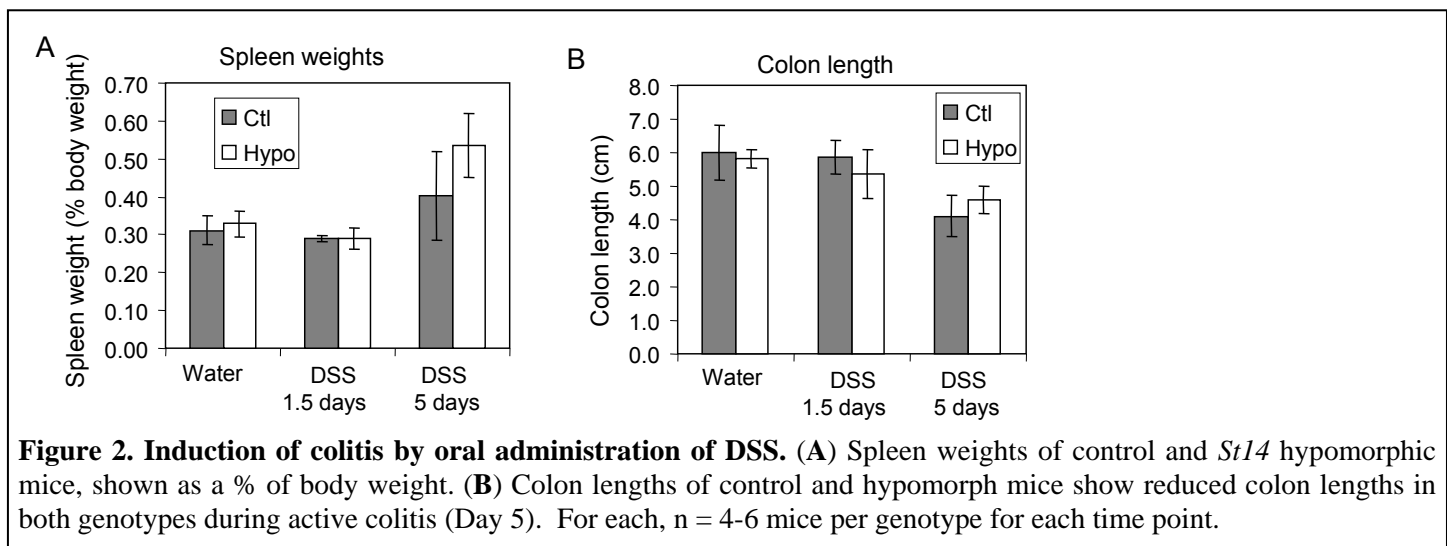
Year 2 progress – During this year we obtained sufficient numbers of mice to expand the time course of treatment of control and *St14* hypomorphic mice with 2% DSS to induce colitis. Due to increased potency of a new batch of DSS, we are treating mice with 2% DSS in drinking water for 5 days to induce comparable symptoms of colitis as observed previously at 7 days. During this past year, we have completed treatment of Day 5 DSS treated mice (11 control, 7 *St14* hypomorphs), Day 1.5 DSS treated mice (4 control, 4 *St14* hypomorphs), and untreated mice (5 control, 4 *St14* hypomorphs). All mice were monitored for disease symptoms and body weight (**Figure 1**), and tissue specimens collected for subsequent molecular analyses. These data confirm our previous findings that matriptase deficiency in mice enhances disease severity.



(b) To sacrifice mice at various time points (3-10/group) during the course of the DSS protocol to evaluate molecular markers of acute inflammation in the subepithelial lamina propria, including quantity and nature of immune cell infiltration, and inflammatory cytokine production (months 1- 18)

Year 1 summary - Preliminary analysis of inflammation associated with the intestinal mucosa in *St14* hypomorph mice was investigated by histological comparison of identical segments of colonic tissue after 2% DSS treatment. Initial analyses of H&E stained tissues of the distal colons for microscopic injury showed a) increased immune cell infiltrates in the mucosa and submucosa in both the *St14* hypomorph and littermate control genotypes after DSS treatment, and b) increased loss of epithelium in the colons of *St14* hypomorph mice compared with their corresponding control littermates, either due to increased epithelial sloughing/shedding or loss due to epithelial cell death. These possibilities will be further examined in Task 2(f). Preliminary analysis of cytokines and inflammatory mediators produced in response to DSS-induced colitis showed a) littermate control mice displayed an increase in $\text{TNF}\alpha$ after DSS treatment, which was not observed in *St14* hypomorph mice, and b) IL-13 in *St14* hypomorph mice increased relative to littermate controls, and this balance appeared to change after DSS treatment.

Year 2 progress - We have continued to perform DSS treatments on increased numbers of control and hypomorph mice (1.5 days and 5 days) to collect tissues for H&E staining, immunohistochemistry for immune cell markers and Q-PCR analyses for cytokine expression. The treatment of mice for 1.5 days will enable analysis of early changes in the initiation of inflammation and immune responses in *St14* hypomorph mice compared to control littermates. Interestingly, while further animal numbers are required, 50% of hypomorph mice showed some clinical symptoms as early as 1.5 days after initiation of DSS treatment, whereas no symptoms are detected in control mice at this time point (**Figure 1**). Initial analyses show a slightly increased spleen size and shortened colon length, in both control and hypomorph mice after 5 days treatment, strongly indicative of an immune response (**Figure 2**). It is expected that hypomorph mice will show a significantly stronger inflammatory response than control mice in studies on the recovery after DSS removal, which are in progress. Analyses of the nature of immune cell infiltration and cytokine expression are also currently in progress.



(c) To sacrifice mice at various time points (3-10/group) during the course of the DSS protocol to assess gut permeability by serum FITC-dextran concentration after gavage and by ex vivo TEER measurements on colonic mucosa mounted transwell chambers. (months 1-18)

Year 1 summary - A 'leaky' mucosal barrier due to decreased barrier function is thought to underlie persistent and destructive mucosal inflammation in IBD and other intestinal disorders. In Year 1, we found that *St14* hypomorphic mice show enhanced baseline intestinal barrier permeability as measured by a) a 20% decreased baseline TEER of the distal colons of *St14* hypomorphic mice (1).

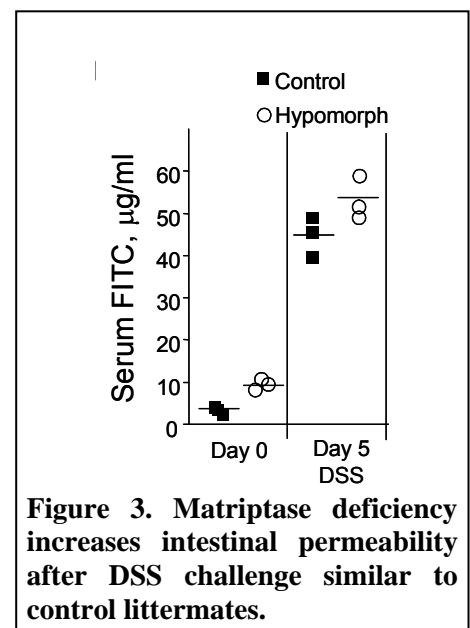
Year 2 progress - We have expanded investigation of barrier permeability to examine intestinal permeability of *St14* hypomorph mice compared with control littermates after DSS challenge. FITC-dextran (4kDa; 500µg/g body weight) was instilled by oral gavage, and the concentration of FITC-dextran in blood collected was measured after 4 or 24 hours in *St14* control and hypomorphic littermates. The results showed that the defective epithelial barrier in *St14* hypomorphic mice contributes to enhanced permeability following DSS insult.

(d) To sacrifice mice at various time points (3-10/group) during the course of the DSS protocol to evaluate bacterial translocation to lymph nodes, spleen and serum. (months 1-18)

We have collected tissue specimens from mesenteric lymph nodes, spleens and colons for analysis of bacterial translocation, and to date have 8 specimens (4 ctl, 4 hypo) from 1.5 day DSS treatment and 10 specimens from 5 day DSS treatment (6 ctl, 4 hypo), along with 9 specimens from water only treated mice (5 ctl, 4 hypo) for comparison. Once a sufficient numbers of specimens from additional time points have been collected, we will measure bacterial translocation by qPCR amplification of bacterial DNA extracted.

(e) To sacrifice mice at various time points (3-10/group) during the course of the DSS protocol to assess differences in claudins and other junctional proteins that have been reported to form a "leaky" barrier in certain tissues between *ST14* hypomorphic and control littermates. (months 1- 18)

Year 1 summary – In preliminary experiments from available mice, tissue segments from identical regions of the jejunum, small intestine, and the colons of littermate control and *St14* hypomorphic mice were collected for analysis of protein and mRNA expression. The initial analysis from a small number of mice showed a) elevated claudin-2 protein expression in *St14* hypomorphs compared to littermate control mice, and b) no change in claudin-2 mRNA expression indicating that regulation of claudin-2 in the mouse colon may be largely post-transcriptional.



Year 2 progress - As for 2(d), we are currently continuing to collect tissue specimens to perform these analyses with all collected samples.

(f) To sacrifice mice at various time points (3-10/group) during the course of the DSS protocol to compare markers of epithelial regeneration in the colonic mucosa of *St14* hypomorphic with control mice, including staining of distal colonic segments for Ki-67, BrdU label incorporation, and epithelial apoptosis (months 6-24)

Year 1 summary - Epithelial regeneration in the colonic mucosa of DSS-treated *St14* hypomorphic mice will be investigated by immunostaining of colonic segments for Ki-67, a nuclear protein preferentially expressed during active phases of the cell cycle and which is absent in resting cells (3), and secondly by injection of BrdU (50mg/kg) prior to sacrifice, which gets incorporated into dividing cells and can be immunostained as a marker of DNA synthesis (4; 5). Our preliminary analysis of colonic tissues from a limited number of untreated control and *St14* hypomorph mice showed strong positive staining for both Ki67 and BrdU by immunostaining, which appeared to be reduced in both genotypes after DSS treatment.

Year 2 progress - We are currently continuing to collect tissue specimens to perform this analysis on increased numbers of mice and at other time points during DSS treatment. We have obtained specimens from 3 *St14* hypomorph and 3 control littermates at 0, 1.5 and 5 days, which are currently being stained for BrdU. Epithelial crypts per mouse will be scored for BrdU-labeled cells and for Ki67 staining, and their position recorded relative to the base of the crypt. We plan to investigate other cell markers and *in situ* cell death by TUNEL assay and activated caspase-3 staining of tissue sections in Year 3.

TASK 3: To define mechanisms responsible for down regulation of matriptase in inflamed mucosa. (months 12-36).

OBJECTIVE: To test the hypothesis that matriptase is down-regulated by cytokine-mediated inflammatory signaling pathways induced by immune system activation.

We and others have shown that loss of matriptase and protein expression disrupts barrier function (1; 2). Thus the mechanisms involved in the down-regulation of matriptase mRNA are of critical importance. The experimental tasks are designed to investigate how and when matriptase is regulated in inflamed mucosa and during barrier recovery using the *in vivo* β -gal reporter gene trap under the control of the matriptase promoter in both heterozygous and hypomorphic *St14* mice.

It was planned to focus on this task in Years 2 and 3 as proposed in the timeline.

The specific tasks are:

(a) To induce colitis in groups of 3-10 *ST14* hypomorphic (-/GT) and control (+/GT) mice by administration of DSS for 5 days in drinking water and then replace with water only. Mice will be monitored for body weights and clinical symptoms (months 12-36)

Year 2 progress - In year 2 we have obtained tissues from Day 5 DSS treated mice (2 control, 4 *St14* hypomorphs), Day 1.5 DSS treated mice (4 control, 4 *St14* hypomorphs), and untreated mice (3 control, 4 *St14* hypomorphs) that will be suitable to detect β -galactosidase reporter activity. This analysis is in progress.

(b) To identify the time-dependent changes in matriptase promoter driven gene expression by X-gal staining, combined with co-staining approaches for molecular marker and cytokine expression. (months 12-18)

Year 1 summary- We found that tissues from untreated control mice (+/GT), that had been fixed with 4% PFA produced negative staining for β -galactosidase activity (Roche kit). Since these tissues are to be used as the positive control for comparison after DSS treatment, optimization of technical conditions was required.

Year 2 progress - After trying several different methods to optimize detection of β -galactosidase activity on colonic tissues fixed in 4% PFA, an optimized X-gal staining protocol was developed using a short (2 min)

fixation in gluteraldehyde on cut frozen sections. Applying this method, strong positive X-gal staining (blue) was observed in frozen sections of colonic tissues from untreated control mice (+/-GT) and hypomorph mice (-GT) (**Figure 4**). This approach was applied at the same time to control and *St14* hypomorph mice that had been treated with DSS for 5 days to examine whether matriptase promotor activity may be down regulated during DSS-induced inflammatory colitis. In this preliminary experiment, after DSS treatment, both control and *St14* hypomorph mice showed a reduction in X-gal staining compared to untreated mice of the same genotype (**Figure 4**), indicating that matriptase is down-regulated at the transcriptional level during DSS-induced inflammatory colitis.

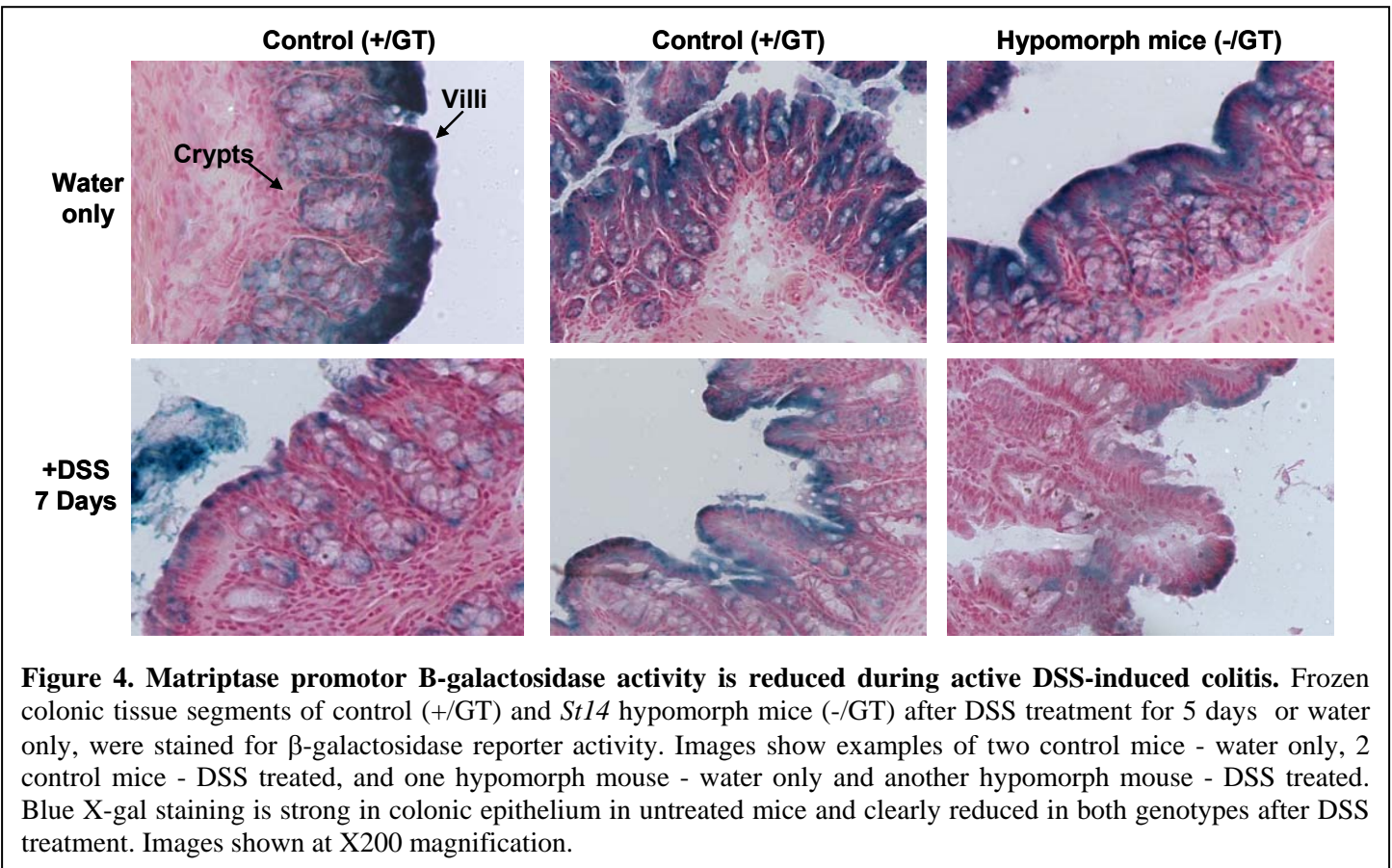
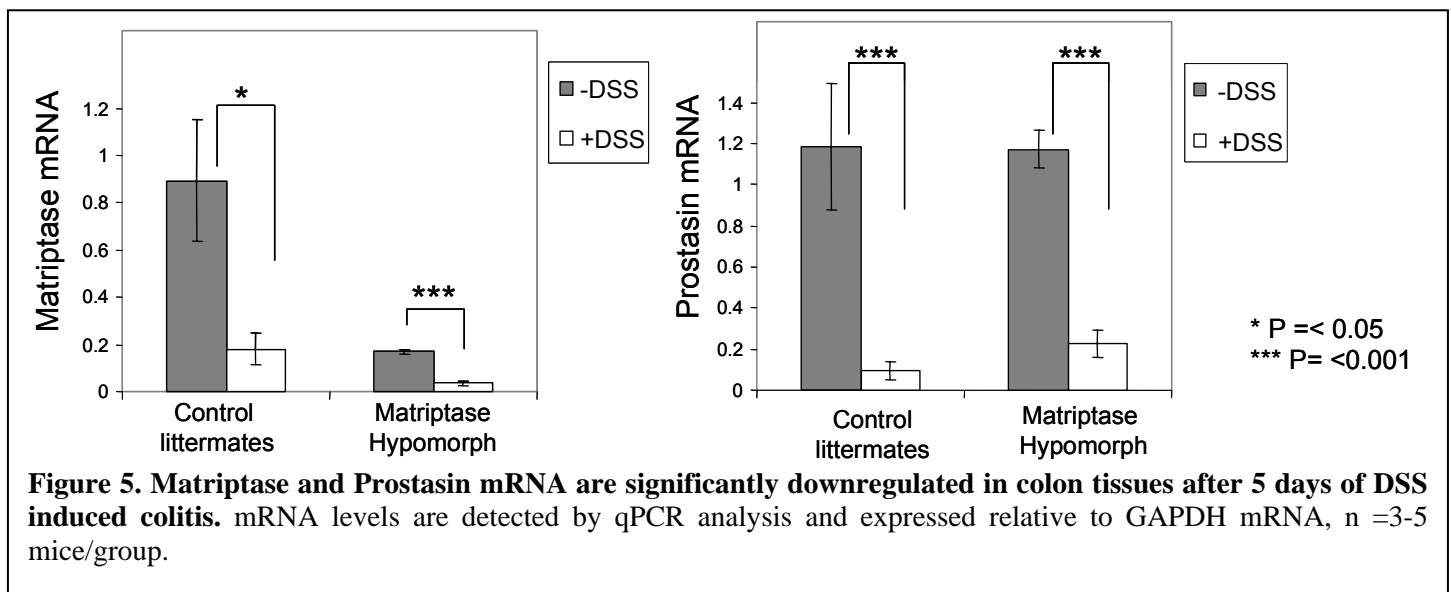


Figure 4. Matriptase promotor B-galactosidase activity is reduced during active DSS-induced colitis. Frozen colonic tissue segments of control (+/GT) and *St14* hypomorph mice (-/GT) after DSS treatment for 5 days or water only, were stained for β -galactosidase reporter activity. Images show examples of two control mice - water only, 2 control mice - DSS treated, and one hypomorph mouse - water only and another hypomorph mouse - DSS treated. Blue X-gal staining is strong in colonic epithelium in untreated mice and clearly reduced in both genotypes after DSS treatment. Images shown at X200 magnification.

(c) To analyze tissue specimens by qPCR for total RNA levels and matriptase protein expression (months 12-24).

Year 1 summary - We initiated qPCR studies to investigate changes in matriptase mRNA expression in the absence and presence of colitis. Preliminary results of a limited number of mice treated with DSS for 7 days showed that matriptase expression is down-regulated in the colonic mucosa of control mice during DSS induced injury. We also found that the related protease prostasin which is important for matriptase activation in intestinal epithelium and essential for matriptase-mediated barrier formation (6), is also down-regulated by qPCR analysis.

Year 2 progress – We have increased mouse numbers and isolated mRNA from colon tissues after 5 days DSS treatment (3-5 mice/group). qPCR analysis for matriptase and prostasin levels show a statistically significant decrease in mRNA levels for both of these proteases during active colitis (**Figure 5**). There is a possibility that the decreased levels of these proteases is a consequence of loss of epithelial cells during active colitis, and this is being investigated by comparative analysis with EpCAM mRNA, an epithelial cell marker. The data shows a decrease in matriptase mRNA, consistent with the observed loss of matriptase promotor-driven β -gal reporter expression (**Figure 4**). Together these data suggest that loss of this barrier forming pathway during colitis occurs at the level of reduced mRNA transcription.



(d) To correlate matriptase gene expression with cytokine profiles to identify effectors of matriptase down-regulation during inflammation. (months 12-24)

In Year 2 tissue collection has been ongoing for this analysis.

(e) To treat mice with antibiotics for a 4 week period prior to DSS challenge. (months 18-36)

To be completed in Year 3

(f) To establish whole colon cultures from - /GT mice and +/GT mice and assess viability and efficacy for ex vivo studies. (months 12-36)

To be completed in Year 3

(g) To investigate specific regulation of the matriptase gene activity by specific inflammatory cytokines and incorporating cytokine pathway inhibitors (months 12-36)

To be completed in Year 3. Data obtained using animal studies (Task 3(d)) and from cell culture systems (Task 4) will be used to guide the selection of inflammatory mediators and their inhibitors to be tested in these experiments.

TASK 4: To test the hypothesis that matriptase loss, induced by IL-13 during inflammation, mediates the IL-13 mediated effects on increased permeability and delayed epithelial barrier repair via derangement of tight junction integrity, increasing expression or localization of claudin-2 or other TJ proteins and stimulation of epithelial cell apoptosis (months 1-18).

OBJECTIVE: To test the hypothesis that matriptase loss, induced by IL-13 during inflammation, mediates the observed IL-13 effects on increased permeability and delayed epithelial barrier repair.

This task aims to identify a possible mechanism by which the IL-13/STAT6 signaling pathway induces apoptosis and barrier disruption. IL-13 production and claudin-2 expression are both increased in human ulcerative colitis and Crohn's disease (7; 8), and matriptase is decreased (1). We will investigate the hypothesis that IL-13 induced down-regulation of matriptase contributes in a significant way to IL-13 dependent barrier dysfunction.

The specific tasks are:

(a) To grow cultures of polarized Caco-2 and T84 cells on permeable transwell supports and treat with IL-13 for various times (months 1-3).

Year 1 summary - We were able to grow T84 cells on permeable filter supports enabling the formation of highly polarized monolayers (TEER > 1500 Ohms.cm²), to obtain initial preliminary data using this cell line. However, we had encountered problems with barrier formation and TEER development in Caco-2 cells, which has now been resolved.

Year 2 progress – We have made significant progress with our analysis of IL-13-mediated barrier disruption and regulation of matriptase in T84 cultures (see below). With regard to Caco-2 cultures, we hypothesized that the reason the Caco-2 cultures were not developing highly polarized monolayers could have been due to differences in the batch of fetal calf serum used in the culture medium. We found that increasing the concentration of serum in the media from 10% to 20% allowed the Caco-2 cultures to develop a strong barrier (Figure 6), as indicated by TEER measurements. Cytokine treatment of these cultures is in progress, in order to confirm the findings we have obtained in T84 cultures.

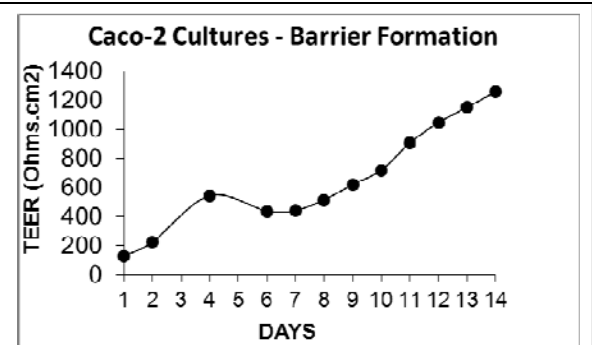


Figure 6. Development of barrier function by Caco-2 cultures. Cells were plated on transwell inserts in media containing 20% FCS, and TEER development monitored.

(b) To monitor the integrity of the barrier by measurement of TEER and by the flux of 4kDa FITC conjugated dextran across monolayers (months 1-3)

Year 1 summary - Preliminary data showed that after treatment of polarized T84 monolayers with IL-13, barrier disruption occurred as monitored by a reduction in TEER and an increase in monolayer permeability to macromolecular FITC-dextran.

Year 2 progress – We have been able to consistently induce barrier disruption of T84 cultures using IL-13 in combination with the related cytokine IL-4, which is also up-regulated in active colitis. IL-4 functions through activation of the same cell surface receptors as IL-13, and activation of the STAT-6 pathway similar to IL-13. **Figure 7** shows an example of barrier formation and cytokine induced barrier disruption in the T84 cultures.

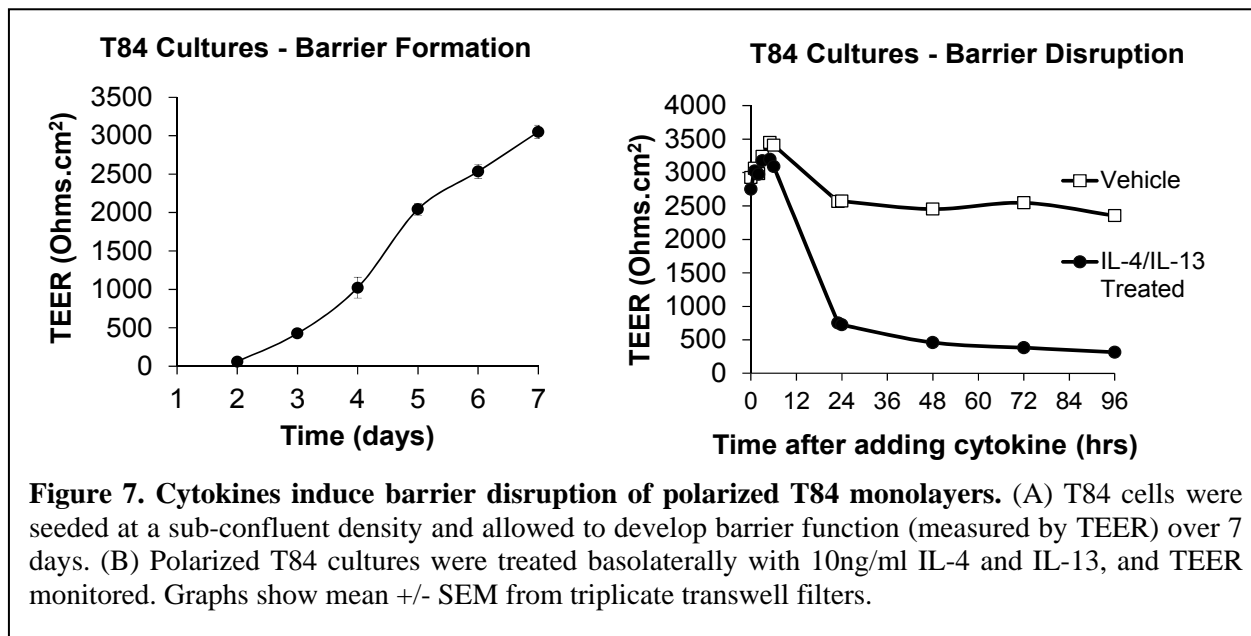


Figure 7. Cytokines induce barrier disruption of polarized T84 monolayers. (A) T84 cells were seeded at a sub-confluent density and allowed to develop barrier function (measured by TEER) over 7 days. (B) Polarized T84 cultures were treated basolaterally with 10ng/ml IL-4 and IL-13, and TEER monitored. Graphs show mean \pm SEM from triplicate transwell filters.

(c) To investigate the effects of the restoration of recombinant matriptase to cell monolayers and determine the effects on IL-13 induced barrier permeability, claudin-2 levels, apoptosis, STAT6 phosphorylation and restitution rate in vitro (months 3-12)

Year 1 summary - Studies focused on establishing a T84 culture system in which barrier disruption and loss of matriptase expression is reproducibly correlated in a time dependent manner. Preliminary studies showed that after the addition of IL-13, matriptase mRNA was down regulated within 6 hours, while claudin-2 expression

was up-regulated as we have observed previously for Caco-2 monolayers. Matriptase protein was lost after 3 days of cytokine treatment, which was the earliest time point assessed at this time. Correlating with this, epithelial STAT6 phosphorylation was observed.

Year 2 progress – We have established T84 culture models showing the time dependent loss of both matriptase and prostasin that occurs during IL-4/IL-13 induced barrier disruption. Analysis of cultures over time shows that the mRNA for matriptase is down regulated within 6 hours and remains low during the course of cytokine treatment (**Figure 8**). Similarly, prostasin mRNA is also rapidly down regulated by cytokine treatment but is almost recovered by 24 hrs (**Figure 8**). These data are consistent with the reduced matriptase transcription observed after DSS-induced colitis *in vivo*.

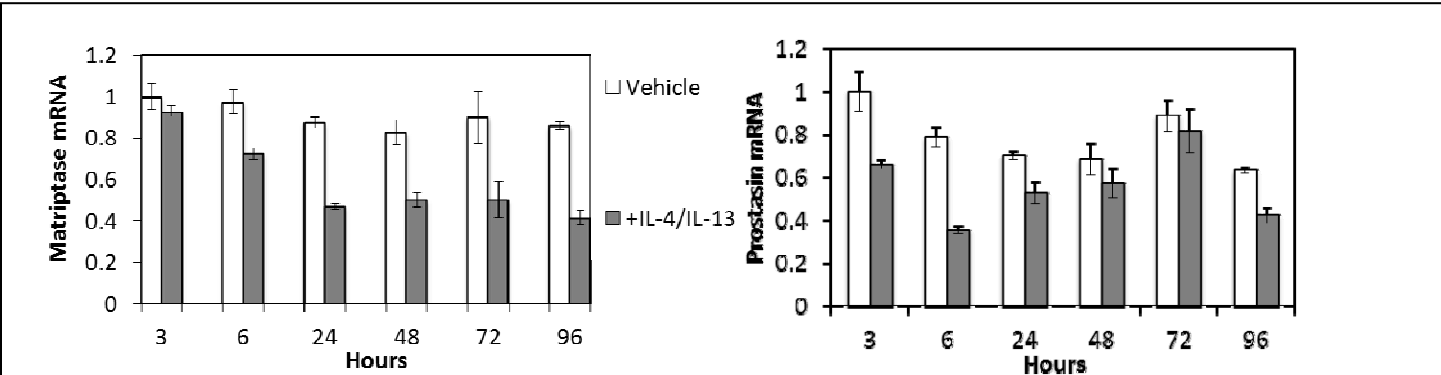


Figure 8. qPCR analysis of polarized T84 cultures treated +/- IL-4/IL-13. Polarized T84 monolayers on transwell filters were treated daily on the basolateral side with 10ng/ml IL-13 or left untreated, for up to 96 hours. Levels of matriptase and prostasin mRNA were analyzed by qPCR. RNA signals are normalized to GAPDH and expressed relative to untreated T84 monolayers.

To examine changes in protein expression, at various times after IL-13 treatment, lysates from parallel cultures were also analyzed for protein expression by immunoblot analyses. We found that a significant reduction in both matriptase and prostasin protein expression can be detected 24 hours after IL-4/IL-13 treatment (**Figure 9**), paralleling the decreases in mRNA expression.

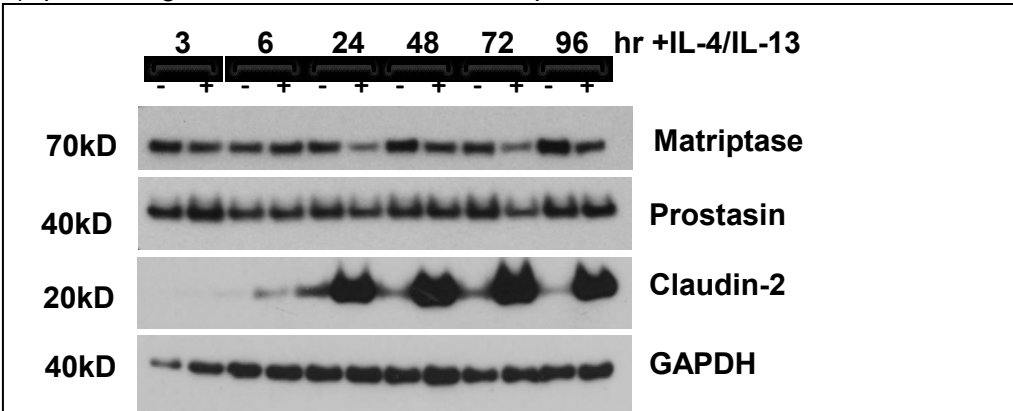
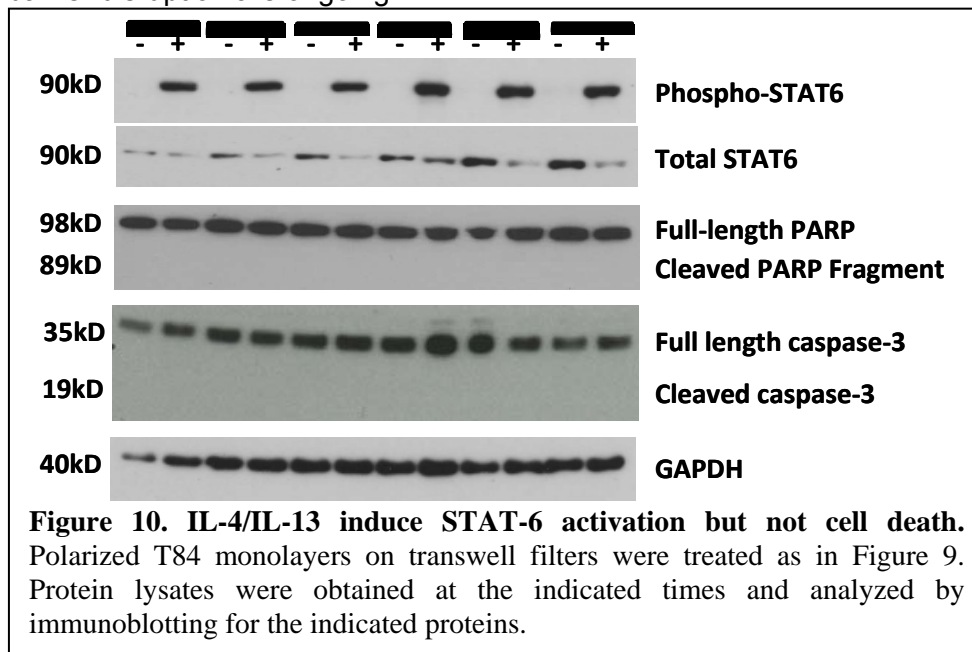


Figure 9. A Reduction in Matriptase and Prostasin protein expression is induced by IL-4/IL-13. Polarized T84 monolayers on transwell filters were treated as in Figure 9. Protein lysates were obtained at the indicated times and analyzed by immunoblotting.

It has been shown that ulcerative colitis is associated with increased colonic epithelial STAT6 phosphorylation, and STAT6 inhibition prevents IL-13-induced apoptosis and barrier disruption (9). We found that treatment of T84 monolayers results in a substantial increase phospho-STAT6 (**Figure 10**), which correlates with reduced matriptase expression and increased permeability of T84 monolayers. We also investigated whether the barrier disruption observed following treatment with IL-4/IL13 is associated with apoptosis of epithelial cells. Analysis of PARP cleavage and the absence of activated caspase-3 in lysates treated for up to 96 hrs show no significant evidence of cell death in the cultures (**Figure 10**). Therefore the barrier disruption is not inducing cell

toxicity. Experiments to investigate the effects of restoration of recombinant matriptase on the IL-13 mediated barrier disruption are ongoing.



(d) To transfect epithelial monolayers with pcDNA plasmid vectors encoding human matriptase and control constructs, generate and characterize stable cell lines, and determine the impact on IL-13 induced barrier permeability, claudin-2 levels, apoptosis, STAT6 phosphorylation and restitution rate in vitro (months 4-18)

Year 1 Summary – Preliminary studies in which SW480 cells (which do not express endogenous matriptase) were transfected with human matriptase cDNA in pcDNA3.1 and vector alone, showed enhanced transmigration across wounded monolayers.

Year 2 Progress - Studies are planned to investigate T84 restitution in the absence and presence of IL-13 in the presence or absence of matriptase. To achieve this, we are designing an expression strategy to allow detection of cells expressing the transfected (ectopic) matriptase, distinct from the endogenous matriptase.

TASK 5: Perform data analysis and prepare technical reports (months 1-36).

We are continuing data analysis, preparation of technical reports and planning publication of the findings.

KEY RESEARCH ACCOMPLISHMENTS:

- Matriptase plays a critical role in epithelial barrier formation and assembly.
- Matriptase deficiency leads to persistent, severe inflammatory DSS-induced colitis.
- Persistent colitis in ST14 hypomorphic mice is associated with an inability to recover TEER.
- Matriptase is down-regulated in intestinal mucosa during experimental DSS-induced colitis.
- Matriptase is down-regulated by IL-13 implicated in the pathogenesis of IBD
- Loss of the prostasin-matriptase barrier forming pathway during colitis occurs at the level of reduced mRNA transcription in both an animal model and in experimental intestinal epithelial monolayers..

CONCLUSION:

Compromised intestinal barrier function is strongly associated with the pathogenesis of IBD. This project seeks to understand a critical new mechanism by which intestinal epithelial barrier function can be compromised during inflammatory colitis that occurs during IBD. The enhanced susceptibility of matriptase hypomorph mice

to DSS-induced injury and inflammatory colitis combined with the *in vitro* assays of barrier function, suggest that matriptase could be an important contributor to inflammatory colitis and repair of injured intestinal mucosa. Our data to date show that matriptase deficiency in mice enhances disease severity and suggest that the nature of inflammation may be altered by the presence and absence of matriptase. These data support the notion that the defective epithelial barrier in *St14* hypomorphic mice likely leads to persistent inflammation following DSS insult. During this second year of the grant, we have focused on obtaining sufficient tissue specimens for detailed molecular characterization of changes induced by DSS colitis and repair processes in *St14* hypomorphic and control mice (Task 2), the *in vivo* regulation of matriptase during inflammatory colitis (Task 3), and completing the studies on the mechanisms involved in IL13 regulation of experimental epithelial barriers and permeability with a focus on the role of matriptase (Task 4). A significant new finding obtained during this past year is that the loss of the prostatic matriptase barrier forming pathway during colitis occurs at the level of reduced mRNA transcription, both *in vivo* and in cell culture models.

PUBLICATIONS, ABSTRACTS AND PRESENTATIONS :

a) Manuscripts:

Netzel-Arnett S, Buzza MS, Shea-Donohue T, Désilets A, Leduc R, Fasano A, Bugge TH, Antalis TM. Matriptase protects against experimental colitis and promotes intestinal barrier recovery. *Inflamm Bowel Dis*. 2012 Jul;18(7):1303-14. PMID: PMC3288858.

Buzza MS, Martin EW, Driesbaugh KH, Désilets A, Leduc R, Antalis TM. Prostatic matriptase is required for matriptase activation in intestinal epithelial cells to regulate closure of the paracellular pathway. *J Biol Chem*. 2013 Apr 12;288(15):10328-37. PMID: PMC3624416

b) Abstracts:

Buzza, MS, Conway, GD, Martin, EW, Shea-Donohue, T, and Antalis, TM. 'Proteolytic Regulation of the Intestinal Epithelial Barrier: Mechanisms and Interventions' 2014 Military Health system Research symposium (MHSRS).

Johnson T, Buzza M, Conway GD, Antalis TM 'Loss of the barrier forming membrane-anchored serine protease Matriptase during gastrointestinal inflammation- potential role in disease pathogenesis. Fifth Annual Cancer Biology Research Retreat, 2014, University of Maryland Baltimore.

Buzza, M.S., Conway, G.D., Martin, E.W., Shea-Donohue, T. and Antalis, T.M. 'Inflammation-induced loss of the Prostatic-Matriptase barrier promoting pathway in intestinal epithelium contributes to the pathogenesis of colitis'. Gordon Research Conference on Plasminogen Activation and Extracellular Proteolysis, Feb 2014, Ventura, CA

Buzza, M.S., Martin, E.W., Driesbaugh, K., Desilets, A., Leduc, R., and Antalis T.M. 'Prostatic matriptase is required for Matriptase activation during the formation and maintenance of the intestinal epithelial barrier'. ASBMB Special Symposia Series, Membrane-Anchored Serine Proteases, Sept 2013, Potomac, MD.

c) Presentations made:

1. Antalis, Toni, 'Proteolytic Regulation of the Intestinal Epithelial Barrier: Mechanisms and Interventions' 2014 Military Health system Research symposium (MHSRS), Harbor Beach Marriott, Fort Lauderdale, Florida, August 18-21, 2014 (Poster)
2. Johnson, Tierra, 'Loss of the barrier forming membrane-anchored serine protease Matriptase during gastrointestinal inflammation- potential role in disease pathogenesis. Fifth Annual Cancer Biology Research Retreat, UMB SMC Campus Center, University of Maryland Baltimore, June 9, 2014 (Poster)
3. Buzza, Marguerite, 'Inflammation-induced loss of the Prostatic-Matriptase barrier promoting pathway in intestinal epithelium contributes to the pathogenesis of colitis'. Gordon Research

Conference on Plasminogen Activation and Extracellular Proteolysis, Feb 2014, Ventura, CA (Oral presentation)

4. Antalis, Toni, 'Membrane-Anchored Serine Proteases in Inflammation and Protease Activator Receptor Signaling' Gordon Conference on Plasminogen Activation and Extracellular Proteolysis, Ventura, CA, February 2014 (Oral presentation)
5. Buzza, Marguerite, 'Prostasin is required for Matriptase activation during the formation and maintenance of the intestinal epithelial barrier'. ASBMB Special Symposia Series, Membrane-Anchored Serine Proteases, Sept 2013, Potomac, MD (Oral presentation)
6. Antalis, Toni, 'Prostasin is required for matriptase activation in intestinal epithelial cells to regulate closure of the intestinal epithelial barrier" XIVth International Workshop on the Molecular and Cellular Biology of Plasminogen Activation, June 4-8, 2013, Notre Dame, Indiana (Oral presentation)

INVENTIONS, PATENTS AND LICENSES : nothing to report

REPORTABLE OUTCOMES: nothing to report

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